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Influence of water presoak on enzyme-retting of flax

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Abstract

Enzyme-retting offers an alternative to the current method of dew-retting to extract fibers from flax (*Linum usitatissimum* L.). Additional steps could improve the efficiency of enzyme-retting and modify the properties of the resulting fibers. Samples of 'Ariane' flax, which were grown in South Carolina during the winter and harvested early for quality fiber or late for both fiber and seed, were presoaked with distilled water before enzyme-retting. Soaked, enzyme-retted, and air-dried fibers were compared with unsoaked, control samples for yield and properties, and the water extract (or a freeze-dried portion) was tested in various methods for its influence on enzyme-retting. Presoaking increased fine fiber yield in some cases, but fiber strength at times was reduced. Analyses of the freeze-dried residue from soaking showed a mixture of sugars (128.6 and 101 mg g^{-1} for early and late harvest, respectively) and aromatic components including *p*-coumaric and ferulic acids and guaiacyl and syringyl units (3.51 and 3.05 mg g^{-1} total aromatics for early and late harvest, respectively). Water extracts from presoaking treatments at 1.0–2.0% (w/v) were not inhibitory to the retting fungus *Rhizopus oryzae* sb or to Viscozyme used for enzyme-retting, based on the Fried test and enzyme activities. Turbidity tests showed slight growth inhibition for *Eschericia coli* and *Streptococcus* sp. in the presence of water extracts from early versus late harvest flax at 0.5% (w/v), with those from late harvest flax more inhibitory. Benefits on the efficiency of water presoaking prior to enzyme-retting were moderate and not uniform in this study, and modifications may depend upon particular flax harvests.

Keywords: Flax fiber; Yield; Enzyme-retting; Aromatics; Microorganisms

1. Introduction

Flax (*Linum usitatissimum* L.) for centuries has provided important industrial products such as textiles, oilseed, and paper/pulp. Fibers from flax, i.e. linen, are reported to be the oldest textile known, dating back to early Egyptian times (Van Sumere, 1992). Recently, flax has been considered a prime source of natural fibers to replace glass in composites (Lepsch and Horal, 1998; Van den Oever et al., 1999). In addition to fiber, flax is the source of linseed oil for industrial uses (Domier,

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1997) and nutritional flaxseeds (Westcott and Muir, 2000).

Fiber flax produced for textiles is grown under precise conditions to optimize fiber quality and harvested prior to full seed maturity (Van Sumere, 1992). In contrast, seed flax is optimized for seed yield and quality, and the fiber, which is from straw residue, is generally shorter, coarser, and lower in quality. The large tonnage of straw residue from the linseed industry (Domier, 1997; Euroflax Newsletter, 2001), and perhaps future increases due to nutritional and health emphases of flaxseeds, constitutes an increasing environmental problem for disposal. Efforts are under way to use larger amounts of the linseed straw for high-value fibers such as those in composites.

Fibers are obtained from flax stems by the process of retting. Retting is usually microbial in nature and occurs through partial degradation of matrix polysaccharides in stems, thus releasing fibers from non-fiber materials (Van Sumere, 1992). Subsequent processing further cleans and refines the fibers. Retting is the major limitation in production of fibers from flax. Until a few decades ago, bundles of flax stems were immersed in water (e.g. rivers, ponds), and fermentation by anaerobic bacteria degraded pectins and matrix components in the plant cell wall, thereby retting the flax (Sharma and Van Sumere, 1992). The fermentation products constituted such a significant ecological problem that water-retting is no longer practiced in western countries. However, research is being carried out to improve water-retting of another bast fiber plant, namely hemp (*Cannabis sativa* L.) (Di Candilo et al., 2000). The method of choice now is dew-retting, where indigenous aerobic fungi colonize and partially degrade the stems in the field (Sharma and Van Sumere, 1992). Dew-retting is limited to geographic regions where temperature and humidity are suitable for fungal activities, and some regions used for linseed production, e.g. Canada and the northern United States (Euroflax Newsletter, 2001), are not conducive to rapid and uniform dew-retting. Perhaps as important as the geographical limitation is the fact that the quality of dew-retted flax fiber is significantly lower than that in water-retted fibers (Sharma and Faughey, 1999).

As a result of poor quality fibers, considerable effort has been expended to improve dew-retting. One method that has been tried over some time is the use of enzymes for retting (Van Sumere, 1992). Use of water-retting tanks for enzyme-retting in immersion trials has produced fibers with properties as good as water-retted samples (Van Sumere and Sharma, 1991). However, cost appears to be one major disadvantage in enzyme-retting in this manner. Recently, we reported (Akin et al., 2000) a spray-enzyme-retting method where mechanical disruption of plants and inclusion of chelators with pectinase-rich enzyme mixtures were employed to make enzyme-retting more efficient. Enzyme amounts were reduced, and fibers of suitable properties from fiber and seed flax were produced in small pilot plant amounts (Akin et al., 2001a). However, the economics of textile and composite industries require the most efficient methods for fiber production, and efforts are being made to optimize the enzyme-retting method such as the most effective means of applying enzymes (Akin et al., 2002; Foulk et al., 2001).

Further research is needed to improve enzyme-retting. A practice previously used for water-retting in Europe was to pre-wash flax to remove contaminating inorganic salts, colored materials, and soil (Sharma and Van Sumere, 1992). Our experience has shown that soaking of flax results in substantial discoloring of water, indicating large amounts of soluble, colored plant products. Because enzyme-retting could also benefit from such a pretreatment, we evaluated water-soaking pretreatment to remove these soluble compounds. The objective of this study was to evaluate water soaking of crimped stems of flax, which was grown for fiber and for fiber plus seed, to remove potential inhibitors and improve enzyme-retting.

2. Materials and methods

2.1. Sample

'Ariane' flax was grown during the winter of 1998/1999 in the coastal plain region of South Carolina and harvested early (May 5) for fiber or late (May 27) for seed and fiber (Foulk et al.,

2000). Cut stems were dried without dew-retting and crimped (Akin et al., 2000) to disrupt stem integrity. Material from these harvests was used in three experiments to test the effect of water soaking prior to enzyme-retting on retting efficiency and fiber properties and evaluate the water extract for detrimental effects on retting.

2.1.1. Presoaked, enzyme-retted flax and antimicrobial properties of water extract (Experiment 1)

Four hundred grams of crimped whole (excluding roots), early harvested flax plants were soaked in distilled water for 24 h at room temperature and then air-dried in a laboratory hood.

Fifty-gram portions of the water-soaked and dried flax stems and 50 g portions of non-soaked plants from the same early harvest were spray-enzyme-retted (Akin et al., 2000) with 0.05% Viscozyme L (Novozymes, Franklinton, NC) plus 1.8% Mayoquest 200 (18.25 mM EDTA) (Callaway Chemical Co., Smyrna, GA) in water, pH 5.0. Approximately 200 ml of enzyme formulation was sprayed onto the 50 g flax samples, and the thoroughly wet stems were left in the excess liquid for 2 min. Flax was drained of excess formulation, placed in plastic storage bags, and incubated at 40 °C. Duplicate samples were incubated for 4, 8, or 24 h. Samples were washed by soaking in a pan of water for 30 s followed by 30 s in running tap water and then air-dried. Retted straw was hand-carded using a Friche Chain Drive Bench Carder (Friche Enterprises, Granite Falls, WA) to remove most of the shive. Hand-carded fibers were then passed through a Shirley Analyzer (SDL America, Inc., Charlotte, NC), which uses a rotating drum and air flow to separate fine fibers from coarse fibers and trash. The yield of this fine fiber, as an assessment of retting efficiency, was calculated based on the amount of hand-carded fiber and on the initial crimped straw. Shirley-cleaned fibers were analyzed for strength and elongation using the Stelometer, based on the methods developed for cotton (ASTM, 1999a), and for fineness using air flow, based on the micronaire (ASTM, 1999b) that was modified to use 5.0 g fiber samples based on calibration with flax fineness standards from the Institut Textile de France, Lille, France (Akin et al., 1999).

The water extract was tested for general antimicrobial effects using a gram-negative bacterium, i.e. *Eschericia coli*, and a gram-positive bacterium, i.e. *Streptococcus* sp., Gp B, ATCC 12386. The extract was filtered through two layers of cheesecloth to remove large particles. Then, water extracts from flax and distilled water as a control were filtered through 0.2 m filter, and 1, 2.5, or 5 ml were added to 7 ml of brain heart infusion medium (BHI No. 0037-01-6, Difco, Detroit, MI) in matched test tubes for turbidity tests. The test tubes were then inoculated with 0.2 ml of 16 h cultures of *E. coli* or *Streptococcus* sp. and incubated at 37 °C. To test Turbidity for duplicate tubes, each treatment was assessed hourly using a Spectronic 21D spectrophotometer (Milton Roy, Hong Kong) at 520 nm.

Extract from the flax was further tested for its ability to inhibit retting as assessed by the Fried test (Henriksson et al., 1997a). About 1 l of water extract was filtered through two layers of cheesecloth and then filtered through a 0.45 m filter. The filtrate was divided into three portions, frozen overnight, and then freeze-dried for several days. The hydroscopic, dark residue was stored in a desiccator until used. The freeze-dried residue was added at 1 or 2% (w/v) to enzyme-retting formulation in 50 mM sodium acetate buffer, pH 5.0, containing 0.05% (v/v) Viscozyme L plus 1.8% (v/v) Mayoquest 200. Similarly prepared tubes, but without flax extracts were included as controls. Twelve 10 cm long segments per tube from the center of Ariane stems were enzyme-retted as described (Henriksson et al., 1997a), and retting efficiency was judged by standard images from 0 = no fiber separation to 3 = complete fiber separation from stems based on the Fried test method described (Henriksson et al., 1997a).

2.1.2. Presoaked, enzyme-retted flax (Experiment 2)

Fifty-gram portions of crimped flax stems from each of early and late harvests of Ariane flax were soaked with 2 l of distilled water as described above. Soaked and unsoaked portions in triplicate were spray-enzyme-retted as above for 24 h. Fiber

yield and properties were determined as described previously. No extracts were tested for anti-retting effects.

2.1.3. Presoaked, enzyme-retted flax and antimicrobial properties of water extract (Experiment 3)

Fifty-gram portions of early and late harvests of Ariane flax were soaked and spray-enzyme-retted for 24 h in triplicate as in Experiment 2. Fiber yields as well as the antimicrobial and anti-retting activities of the water extracts were determined. About 2 l of water extract was centrifuged at 1500 rpm for 30 min. The supernatant was filtered through a glass fiber filter (3 μm), then a glass microfiber filter (1.2 μm), and finally a 0.45 μm filter. The filtrate was divided into portions and freeze-dried as in Experiment 1, and the hydroscopic residue was stored in a desiccator until used.

The inhibitory effects of extracts from the early and late harvested flax were tested by various means. The general antibacterial effect was tested at 0.5% (w/v) extract residue using the two organisms described in Experiment 1 and identical procedures. The antifungal effect was tested using *Rhizopus oryzae* sb isolated from flax dew-retted in South Carolina (Henriksson et al., 1997b; see Akin et al., 2001b for corrected identification). Mycelial disks about 5 mm in diameter from an actively growing culture on 0.2% (w/v) citrus pectin (9135, Sigma, St. Louis, MO) in Vogels medium (Henriksson et al., 1997b) were inoculated onto similar pectin plates with 0, 0.5, or 1.0% (w/v) of each flax extract residue. The diameter of the mycelium was measured in two directions at right angles to each other for triplicate plates each treatment after 7 and 24 h.

Enzyme inhibition by freeze-dried residues of extracts was assessed using the dinitrosalicylic (DNS) acid assay (Miller, 1959) for release of reducing sugars from polygalacturonic acid. Viscozyme L and *Aspergillus niger* pectinase (31660, Serva, Heidelberg, Germany) were tested using 10 μl of a 10^{-4} and 10^{-3} dilution, respectively. Release of reducing sugars was measured (four replicates each) with 0, 1.0, 0.1, 0.01, or 0.001% (w/v) of freeze-dried extracts from early or late harvested flax. Results are reported as μmoles sugar released per min per ml.

The freeze-dried extracts from soaked plants of early and late harvested flax were analyzed for chemical components. Solid residues were treated with 4 M NaOH for 2 h at 170 °C, adjusted to pH 2.5 with 2 M HCl, and extracted with ethyl ether. Sample and base were heated in a screw-capped Teflon vial. The solution was purged with nitrogen, and the vial capped and placed in a steel reaction vessel containing 7 ml water. The vessel was sealed and placed in an oven at 170 °C for 2 h. After acidification and extraction with diethyl ether, the ether layer was dried and the residue treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Analyses of aromatics, fatty acids, and alcohols were carried out as previously described (Morrison et al., 1999) on a Finnigan 9001 gas chromatograph using a 0.25 mm i.d. × 30 m DB 5 capillary column (0.25 μm film thickness). Cell wall sugars were analyzed as their alditol acetates by gas chromatography according to the method of Hoebler et al. (1989) with the initial digestion time increased from 30 to 90 min at 40 °C. The column used was a 0.25 mm i.d. × 30 m DB 225 capillary column (0.15 μm film thickness).

3. Results

3.1. Experiment 1

For the large study involving 400 g of flax, results comparing soaked and unsoaked samples prior to enzyme-retting are shown in Table 1. Fine fiber amounts were obtained by passing hand-carded fiber through the Shirley Analyzer. Calculations for the yield of fine fiber from the initial crimped straw weight were adjusted to take into account the loss of soluble material during soaking (ca. 16%). Within incubation times of a treatment, no differences occurred in fine fiber yield based on hand-carded fibers or original crimped straw weight. A 4 h incubation was sufficient to extract enzyme-retted fibers from this flax sample. Using the adjusted weight procedure for fiber calculations from the initial straw, the soaked samples

Table 1
Yield and properties of water-soaked versus unsoaked flax fiber from early Ariane prior to enzyme-retting for 4, 8, or 24 h (Experiment 1)

Treatment[a]	Retting time (h)	Shirley yield[b] (%)		Strength[c] (g tex^{-1})	Elongation[c] (%)	Fineness[d]
		Hand-carded	Straw			
Unsoaked	0[e]	17.5	7.4	37.9±6.8	0.8±0.7	ND[f]
Unsoaked	4	35.1±2.2 ab	16.3±1.0 a	40.9±7.3 a	0.9±0.1	7.9±0.1 a
Unsoaked	8	33.8±0.6 a	15.1±0.6 a	34.5±0.1 ab	0.9±0	7.7±0.4 a
Unsoaked	24	42.7±2.1 bc	16.7±0.3 a	28.8±1.8 bc	0.9±0.1	6.6±0.2 bc
Soaked	0[e]	30.1	16.3	44.0±3.2	1.6±0.6	ND[f]
Soaked	4	45.4±3.1 c	19.6±1.1 a	36.5±3.7 ab	0.9±0.5	7.1±0.1 b
Soaked	8	48.4±1.4 c	20.1±0.1 a	32.2±1.1 bc	1.1±0.4	6.6±0.2 bc
Soaked	24	46.3±5.9 c	17.5±2.7 a	23.9±1.5 c	0.5±0.4	6.3±0.2 c

Values within columns with different letters are significantly different at $P \leq 0.05$.

[a] Fifty-gram aliquots taken from water-soaked overnight or control, unsoaked crimped Ariane flax stems (SC 99) were retted in duplicate by soaking 2 min in 0.05% Viscozyme L plus 18.3 mM EDTA (from Mayoquest 200) in water at pH 5.0 and 40 °C.

[b] Shirley-cleaned fibers 1 × as % of hand-carded sample or based on weight of straw. For control, unsoaked samples, 50 g was used to calculate yield; for water-soaked samples, 58.1 g was used as starting weight, since 16.25% of material had been solubilized during water extraction.

[c] Average±SD of two replications each consisting of six tests by Stelometer.

[d] Average±SD of two replications each consisting of two tests by airflow (Micronaire) method. Some samples 25 g.

[e] Non-retted control consisting of one replication.

[f] Not determined.

tended to yield more fine fibers than unsoaked samples at each incubation time, although differences were not significant ($P > 0.05$). For calculations from hand-carded samples, fine fiber yield was greater ($P \leq 0.05$) for soaked samples at 4 and 8 h incubation and tended to be higher at 24 h. However, mass of non-fiber materials present in the unsoaked samples could have increased the weight differences with soaked samples and thereby influenced this comparison. From a tactile sense, the fibers often appeared softer in water-soaked versus unsoaked samples, but this property was not objectively measured. Fiber strength and fineness of soaked samples were lower than those from unsoaked samples (Table 1). Further, within each of the soaking treatments the fibers were significantly weaker and finer with increased incubation times.

The yield of freeze-dried material, derived from a portion of the extracting water, was 2.1 mg/ml of a brown, hydroscopic residue. Extracts of 1, 2.5, or 5.0 ml per 7 ml medium (0.03, 0.08, 0.15%, w/v) tested on *E. coli* and *Streptoccocus* sp. (Fig. 1a and b) indicated little effect on growth rate or final turbidity over that with equal amounts of water added to medium as a control. Final turbidity was less in *E. coli* with 5 ml of extract, whereas both growth rate and final turbidity were less with 5 ml of water or extract in *Streptococcus* sp. Generally, toxic effects of these levels of extract to the bacteria were little to none.

Addition of the freeze-dried extract to enzyme-retting formulations up to 2% showed no negative effect of retting on intact stem sections with Viscozyme by the Fried test (Table 2).

3.2. Experiment 2

Fine fiber yields calculated from hand-carded fibers and also from the initial, crimped stems indicated no difference ($P > 0.05$) in yield between treatment or between harvest time, although yields from soaked, late harvest flax tended to be higher (Table 3). However, soaking of early harvest flax resulted in a weaker ($P \leq 0.05$) fiber than that from unsoaked samples (Table 3). Fiber properties were similar for soaked and unsoaked late harvest samples.

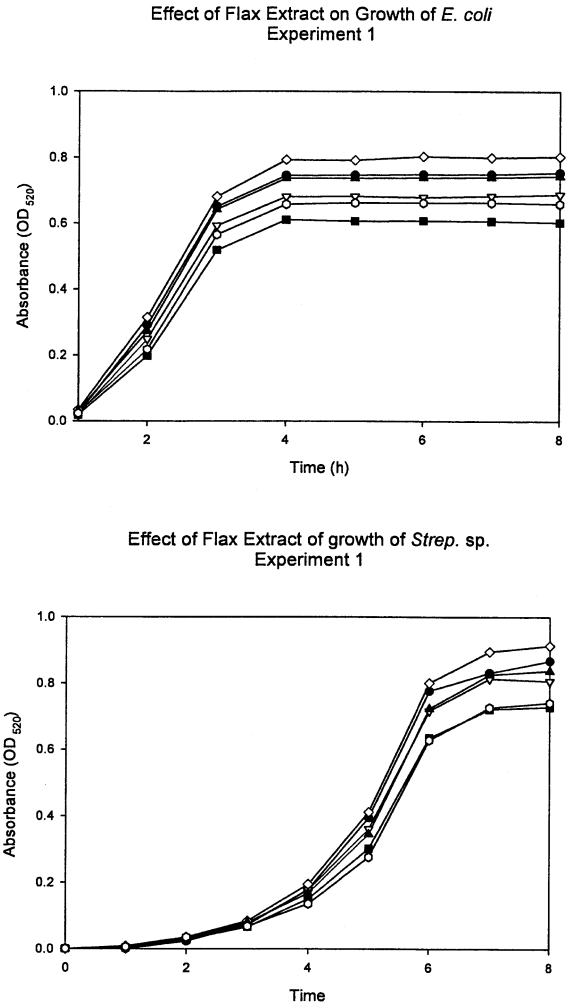

Fig. 1. Growth rate of bacteria in the presence of water extract from early Ariane flax harvest (Experiment 1) (a) *E. coli* and (b) *Streptococcus* sp. ATCC 12386: (●) 1 ml extract; (◇) 1 ml water (control); (▽) 2.5 ml extract; (▲) 2.5 ml water (control); (■) 5 ml extract; □ 5 ml water (control).

3.3. Experiment 3

Yields of the soaked versus unsoaked flax were calculated from crimped, initial straw that was passed three times through the Shirley Analyzer, and comparisons were made after one pass and all three passes (Table 4). Even though the values differed for samples of similar harvests between Experiments 2 and 3, the trends were similar. Fine fiber yield from soaked late harvest flax was higher than from unsoaked, late harvest or either early harvest flax treatment (Table 4). Further, fiber amounts were higher after three passes but in a similar ratio to that after one pass, indicating the samples were not disrupted by soaking so as to be more easily separated during further mechanical processing.

Table 2
Fried test ratings[a] of flax stems incubated with water extract from flax (Experiment 1)

Treatment	Fried test rating[b]	
	Scorer number 1	Scorer number 2
0.05% Viscozyme+18.3 mM EDTA+2% extract	3±0	2.5±0.6
0.05% Viscozyme+18.3 mM EDTA+1% extract	3±0	3.0±0
0.05% Viscozyme+18.3 mM EDTA+0.5% extract	3±0	2.8±0.5
0.05% Viscozyme+18.3 mM EDTA	3±0	2.3±0.5
Buffer+18.3 mM EDTA	0±0	0±0

[a] Rankings are from 0 = no fiber separation to 3 = full fiber separation.
[b] Average±SD of five tubes rated by two scorers.

The brown, hydroscopic residue collected by freeze-drying a portion of the water extract gave yields of 2.0 and 2.2 mg/ml for the early and late harvested flax, respectively, which were similar to the data in Experiment 1. These freeze-dried extracts from early and late harvests were tested at 0.5% (w/v) for the influence on growth of both *E. coli* and *Streptococcus* sp. (Fig. 2). At this level, some general inhibitory effect on growth of the bacteria occurred. In *E. coli*, the logarithmic growth rate was not affected, but overall turbidity was less with the extracts and more so with that from the late harvest extract. In *Streptococcus* sp., both growth rate and final turbidity were substantially reduced, with effects by the late harvest flax extract more pronounced.

Residues of water extracts were further tested for inhibition of a dew-retting fungus and activities of retting enzymes. Radial growth on pectin medium of *R. oryzae* sb was not reduced ($P > 0.05$) from controls in the presence of 0.5 or 1.0% (w/v) extracts from early or late harvested flax. Although growth in the presence of early harvest

Table 3
Fiber yield and properties of flax water-soaked versus unsoaked flax prior to enzyme-retting (Experiment 2)

Sample[a]	Soak time[b] (h)	Shirley-cleaned yield[c] (%)		Strength[d] (g tex^{-1})	Elongation[d] (%)	Fineness[e]
		Hand-carded fiber	Initial crimped straw			
Early harvest	18	37.6±2.3 a	13.0±1.6 a	22.0±0.2 a	0.6±0.1 a	5.0±0.3 a
	0	36.8±3.3 a	13.3±1.2 a	26.4±1.0 b	0.7±0.1 a	5.0±0.1 a
Late harvest	18	38.1±2.7 a	14.2±0.7 a	20.5±0.9 a	0.4±0.4 a	5.5±0.2 a
	0	32.8±4.9 a	12.7±1.6 a	21.1±1.4 a	0.2±0.2 a	5.7±0.3 a

Values within columns with different letters are significantly different at $P \leq 0.05$.
[a] Ariane (SC 99) harvested early for optimal fiber quality and late for mature seed and fiber.
[b] Triplicate samples of 50 g of crimped flax stems soaked in distilled water about 18 h at room temperature and then air-dried or not soaked as controls. Samples then retted with Viscozyme L (0.05%)+18.3 mM EDTA from Mayoquest 200.
[c] Yields from clean fiber obtained by passing enzyme-retted, hand-carded fiber through a Shirley Analyzer (SDL, Charlotte, NC) 1×.
[d] Average and standard deviation of three replicates, each replicate an average of six tests by Stelometer.
[e] Fineness determined by readings based on Micronaire but modified using flax calibration standards (Institut Textile du France, Lille) and 5.0 g samples. Average and standard deviation of three replicates, each replicate an average of two tests.

Table 4
Fiber yield prior to enzyme-retting water-soaked versus unsoaked flax (Experiment 3)

Sample[a]	Soaking time[b] (h)	Shirley-cleaned yield[c] (%)	
		One pass	Three passes
Early harvest	24	19.7±1.5 a	23.0±1.0 a
	0	19.4±0.7 a	23.0±0.6 a
Late harvest	24	24.7±1.4 b	28.0±0.7 b
	0	19.9±1.4 a	24.4±1.4 a

Values within columns with different letters are significantly different at $P \leq 0.05$.
[a] Ariane (SC 99) harvested early for optimal fiber quality and late for mature seed and fiber.
[b] Triplicate samples of 50 g of crimped flax stems soaked in distilled water about 24 h at room temperature and then air-dried or not soaked as controls. Samples then retted with Viscozyme L (0.05%)+18.3 mM EDTA from Mayoquest 200.
[c] Yields from clean fiber obtained by passing enzyme-retted, hand-carded fiber through a Shirley Analyzer (SDL, Charlotte, NC) 1× and residue two additional times.

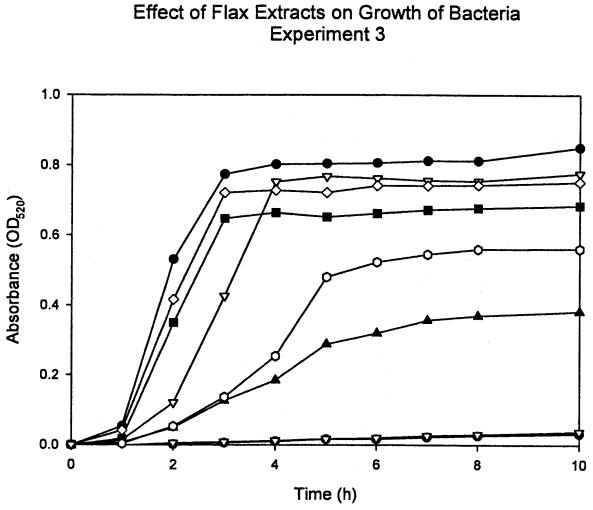

Fig. 2. Growth rate of *E. coli*, *Streptococcus* sp. ATCC 12386 in the presence of water-freeze-dried extract residues (0.05%, w/v) from early and late Ariane flax harvest (Experiment 3): (●) *E. coli* control; (◇) *E. coli*+early harvest extract; (■) *E. coli* and late harvest extract; (▽) *Streptomyces* sp. control; (○) *Streptomyces* sp. and early harvest extract; (▲) *Streptomyces* sp. and late harvest extract (horizontal lines); (▼) early harvest extract control; (□) late harvest extract control.

extracts was not significantly different than from growth without extract, it tended to be greater, with a diameter of the growth zone after 7 h of 24.5, 24.0, and 21.5 mm, respectively, for 0.5, 1.0, and 0%. Mycelial development appeared greater and denser upon microscopic inspection of agar plates with extract than without, showing that this retting fungus was not inhibited by the extracts but instead was slightly stimulated. Pectinase activity, measured by the release of reducing sugars, was complicated by the large amount of free sugars in the extract (Table 5). With both Viscozyme and *A.*

Table 5
Effect of water extracts from early or late harvested Ariane against Viscozyme L or pectinase (Experiment 3)

Extract		Enzyme activity[a] (μmol min^{-1} ml^{-1})	
	Amount (%)	Viscozyme L[b]	Pectinase[c]
Control (No extract)	0	6336	59
Early harvest	1.0	123748	10724
	0.1	16541	1775
	0.01	9431	1411
	0.001	5710	1039
Late harvest	1.0	84955	8757
	0.1	15546	1385
	0.01	11310	869
	0.001	15141	758

[a] Average of four tests per dilution using polygalacturonic acid (1.0%).
[b] Viscozyme L (Novozymes, Franklinton, NC).
[c] Pectinase from *Aspergillus niger* (3L660, Serva, Heidelberg, Germany).

niger pectinase, reducing sugars decreased proportionately with increased dilutions of extract from early flax. With late harvest flax, the amount of reducing sugars was proportionately greater in the higher dilutions, suggesting a slight stimulation. While these data are difficult to interpret because of extraneous sugars, the activities of various dilutions did not suggest an enzyme inhibition by either of these extracts.

Chemical analyses of the brown, hydroscopic residue for the freeze-dried extracts indicated a variety of sugars (Table 6) and aromatic components (Table 7). Waxes and cutins were not detected. Variations between harvests occurred for some sugars, particularly with higher levels of glucose in early harvest, and total levels of these sugars were higher ($P \leq 0.05$) in early harvest flax (Table 6). For aromatics, levels of *p*-coumaric acid and ferulic acid were higher in early harvest stems, but total amounts of soluble aromatics were similar between harvests (Table 7).

4. Discussion

Removal of inorganic salts, colored materials, and soil by a water rinse prior to water-retting was practiced in Europe (Sharma and Van Sumere, 1992). Our results presented herein indicated that water-soaking flax prior to enzyme-retting resulted in a modest improvement of fine fiber yield, but results varied among samples. Further assessments of water presoaking for particular harvests might prove to be economically beneficial. However, clearly fiber strength was reduced in the early harvest of Ariane by this treatment and the influence on fiber quality for particular applications should be considered. While not evaluated in this study, flax wet from water presoak could potentially be more efficiently enzyme-retted due to the rapid and uniform distribution of enzymes.

Compositional analyses of flax tissues have shown that stems have high levels of aromatics, including both guaiacyl and syringyl groups as well as small amounts of phenolic acids (Akin et al., 1996). Solid-phase ^{13}C NMR indicated that

Table 6
Analysis of sugars in water extracts released during soaking of flax stems[a]

Flax[b]	Component[c] (mg g^{-1})						
	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose	Total
Early harvest	22.1±1.3 a	12.4±0.4 a	3.9±0.2 a	17.7±0.7 a	16.4±0.4 a	56.2±2.5 a	128.6±5.3 a
Late harvest	22.9±0.7 a	16.9±0.6 b	5.5±0.1 a	11.8±0.8 b	15.1±0.4 b	28.9±0.3 b	101.1±2.9 b

Values within columns with different letters are significantly different at $P \leq 0.05$ by the *t*-test.
[a] Extract from soaking 50 g portions of crimped flax stems in 2 l water. Liquid then freeze-dried and residue analyzed for chemical components.
[b] Ariane flax grown during winter months in South Carolina and harvested early for fiber or later for fiber and seed.
[c] Average±S.D. for duplicate samples from gas chromatographic analysis.

Table 7
Analysis of aromatic components in water extracts released during soaking of flax stems[a]

Flax[b]	Component[c] (mg g^{-1})				
	p-Coumaric acid	Ferulic acid	Guaiacyl units	Syringyl units	Total
Early harvest	0.40 ± 0.08 a	1.41 ± 0.27 a	0.95 ± 0.2 a	0.74 ± 0.12 a	3.51 ± 0.66 a
Late harvest	0.18 ± 0.04 b	1.05 ± 0.26 b	1.03 ± 0.2 a	0.79 ± 0.24 a	3.05 ± 0.72 a

Values within columns with different letters differ at $P \leq 0.05$ by the *t*-test.
[a] Extract from soaking 50 g portions of crimped flax stems in 2 l water. Liquid then freeze-dried and residue analyzed for chemical components.
[b] Ariane flax grown during winter months in South Carolina and harvested early for fiber or later for fiber and seed.
[c] Average ± SD for triplicate samples from gas chromatographic analysis.

aromatic material in flax fibers was predominately an anthocyanin, rather than lignin (Love et al., 1994). Extraction of flax bast tissue with a series of organic solvents (i.e. hexane, propanol, and methanol) and analysis by reverse-phase HPLC and ^{13}C NMR showed that a variety of aromatic constituents including flavonoids and hydroxy-methoxy cinnamic acids occurred in flax (Gamble et al., 2000). Results presented herein indicate that the water extract from these flax samples is a complex mixture of compounds, including sugars and aromatics representative of the type found in intact plants (Akin et al., 1996).

It is well established that aromatic compounds such as lignin in plants inhibit microbial degradation of lignocellulosic materials (Eriksson et al., 1990). Low molecular weight aromatics, e.g. phenolic acids and phenolic aldehydes, inhibit microbial activity not by physical protection, as occurs with lignin, but by toxicity to both anaerobic and aerobic microorganisms (Borneman et al., 1986; Sethuraman et al., 1998). Further, the interactions of microbes and aromatic compounds are complex and lead to a variety of responses, even stimulation of activity in some cases. However, particular compounds and concentrations are noteworthy in their inhibitory effect on cell wall degrading enzymes (Martin and Akin, 1988). High levels of aromatic components, particularly with the amount and diversity in the flax core cells (Akin et al., 1996), suggest the possibility of enzyme inhibition during enzyme-retting. Specific to the objective in this study on flax, chemical extracts that were particularly high in aromatic content inhibited cellulase and pectinase activities (Gamble et al., 2000).

Despite the presence of aromatic components, generally water extracts from these flax plants did not appear to be particularly toxic to most biological systems tested. *R. oryzae* sb is a major component of the dew-retting fungal consortium isolated from South Carolina (Henriksson et al., 1997b) and contains high levels of endopolygalacturonase (Henriksson et al., 1999; Akin et al., 2001b). Growth of this fungus was not inhibited by up to 1% of the freeze-dried extract, and in fact growth tended to be greater and mycelium denser at times, likely due to the additional sugars presence in the extract. Further, pectinase activities did not appear to be inhibited at 1% levels of the freeze-dried extract, but the additional sugars complicated assessment in this assay. Fiber separation from stems, as judged by the Fried test (Henriksson et al., 1997a), indicated no inhibition by the presence of the extract. However, a substantial inhibition occurred for *Streptococcus* sp. with 0.5% of the freeze-dried extract in both growth rate and final turbidity (as a measure of cell mass). The extract from the mature harvest was more inhibitory than that from the early harvest, but the current analyses do not explain this greater inhibition since late harvest stems had less of potential phenolic acid inhibitors. The identities of such inhibitory substances are unknown.

5. Conclusions

Water soaking of flax as a means to improve enzyme-retting did not provide clear benefits with these samples. Improvement in fine fiber yield occurred at times, but fiber strength was decreased. With both water-soaked and unsoaked early Ariane flax, fine fiber yield was similar for 4, 8, and 24 h incubations, whereas fiber strength and fineness decreased with increased incubation time. Results with these samples indicated that enzyme-retting was as efficient at 4 h as 24 h for yield and suggested that various incubation times could be employed to modify fiber properties. In contrast to the toxicity previously reported for chemical extracts from flax, water extracts from soaked flax, even as a concentrated freeze-dried residue, did not inhibit microbial or enzyme systems related to retting up to 0.5% (w/v). General growth inhibition occurred for the gram-positive bacterium *Streptococcus* sp. with a more pronounced effect with water extracts from mature plants. This inhibition did not appear to be related to aromatic compounds analyzed in this study. The moderate and variable benefits of presoaking in this study suggest the need for sampling a broader range of harvests. At that time, presoaked and wet samples, rather than dried samples, could be evaluated for improved retting efficiency.

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